

IDENTIFYN RESOLUTION STEPDOWN

PARP1

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn™ SRM Alexa Fluor™ 647

WESTERN BLOT -> WIDEFIELD -> WIDEFIELD SIM / APOTOME -> CONFOCAL -> AIRYSCAN
SIM -> SIM² -> SINGLE-MOLECULE STORM

8

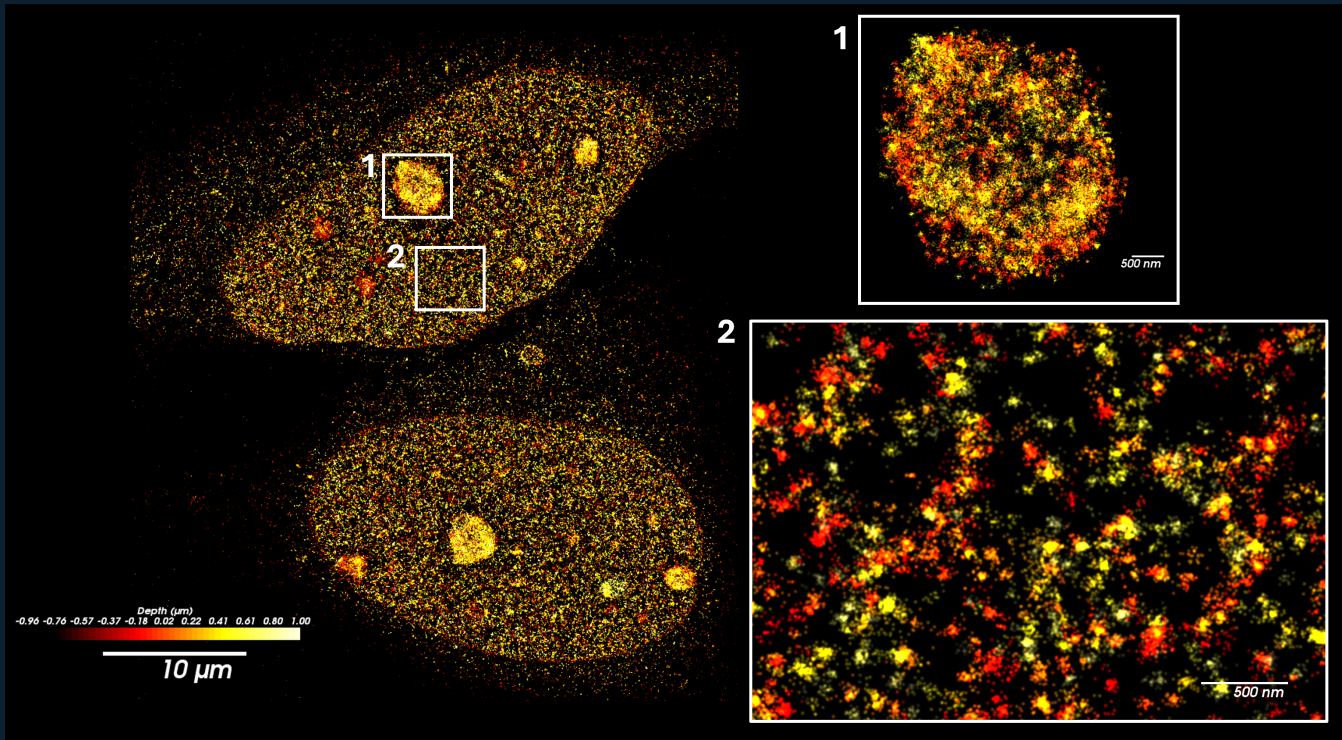
ORDERED EVIDENCE TIERS

0

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

DC-000013 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

CRO CAPABILITY DEMONSTRATED

Identifyn performed the experimental and imaging work documented below. BENNETT converts that operating history into a governed, source-bounded scientific record.

- Source-documented sample preparation and antibody/reagent workflow
- Western blot imaging
- Widefield fluorescence microscopy
- Widefield structured illumination / Apotome optical sectioning
- Confocal microscopy
- Airyscan acquisition and reconstruction workflow
- Structured illumination microscopy
- SIM² reconstruction workflow
- Single-molecule STORM presentation workflow
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for PARP1, and what is the strongest interpretation allowed by the available modalities and controls?

EVIDENCE AVAILABLE

Western blot -> Widefield -> Widefield SIM — Apotome -> Confocal -> Airyscan -> SIM -> SIM² -> Single-molecule STORM

BENNETT ASSESSMENT

The preserved DC-000013 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

SOURCE STATE	SOURCE HASH VERIFIED / EVENT RECORDED
BENNETT EVIDENCE REVIEW	COMPLETED / SOURCE-BOUNDED
NATIVE IMAGE REPROCESSING	NOT PERFORMED
LITERATURE SOURCES	INDEPENDENTLY VERIFIED
SCIENTIST REVIEW	PENDING
PUBLICATION	NOT AUTHORIZED

OVERALL CALL / FULL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved DC-000013 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

SETTINGS ASSESSMENT

The record preserves 8 ordered evidence tier(s). Structured source metadata values are reported by stage and remain tied to the original method objects.

BIOLOGICAL SUPPORT

The record supports retrospective, source-bounded microscopy review. Target identity, specificity, treatment effect, binding, interaction and mechanism are not inferred from presentation images alone.

CONTROL ASSESSMENT

No separate control record is preserved; none is inferred from method or template language.

CLAIM CEILING

This draft supports historical capability documentation, source registration, modality ordering, settings review and bounded interpretation. It does not independently validate antibody specificity, quantify a population response, establish binding, interaction, causation or mechanism, or authorize publication.

PROCESSING DISCLOSURE

Retrospective BENNETT architectural evaluation of preserved Identifyn source evidence. BENNETT did not perform native-file reprocessing, new deterministic measurement or the historical image post-processing represented in this record.

REAGENTS AND ACQUIRED CHANNELS

Preparation reagents are reported separately from channels actually documented in the acquisition settings. Fluorophores mentioned in staining are not automatically treated as acquired channels.

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	647	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 647	3; 50 Cy 5; 38 eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Widefield SIM — Apotome	405/DAPI; 488; 647	3; 50 Cy 5; 38 HE eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Confocal	405/DAPI; 488; 647	2; None; 639 nm @0.09%; 405 nm @0.2%; 653; 353; 668; 465
Airyscan	405/DAPI; 488; 647	2; None; 639nm @2.00%; 405 nm @0.2%; 653; 353; 668; 465
SIM	405/DAPI; 488; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 640
SIM ²	405/DAPI; 488; 647	3; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820-SR; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR; 640
Single-molecule STORM	647	Both Channels

MODALITY ASSESSMENT / PART 1 OF 8

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Azure Sapphire FL. Objective: not explicitly stated. Platform: Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s). Structured source metadata values: 11.

VISIBLE OBSERVATION

A preserved presentation image is available for Western blot. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Band position and displayed intensity do not independently establish analytical specificity, target identity, linearity, or treatment effect.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Western blot evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 2 OF 8

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye: Acquisition: Channels: 3; Scaling (Per Pixel): 0.110 μm X 0.110 μm ; Image size (Pixels): 1216 X 1028; Image Size (Pixels): 1216 X 1028; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 150 ms; 20 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25.00%; X-Cite Xylis Lamp Intensity @15.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 37.

VISIBLE OBSERVATION

A preserved presentation image is available for Widefield. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

A presentation image supports gross localization and source-bounded co-occurrence, not direct binding, molecular colocalization, or native quantitative measurement.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Widefield evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 3 OF 8

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 92 Slices (9.1 μm); Channels: 3; Scaling (Per Pixel): 0.143 μm X 0.143 μm X 0.100 μm ; Image size (Pixels): 1037 X 808; Image Size (Pixels): 1037 X 808; Bit Depth: 14 Bit. Detector/camera: Imaging Device: 807 mono. Timing: Exposure Time: 300 ms; 100 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15.00%; X-Cite Xylis Lamp Intensity @10.00%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 46.

VISIBLE OBSERVATION

A preserved presentation image is available for Widefield SIM — Apotome. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Improved optical sectioning does not by itself establish reconstructed super-resolution or molecular interaction.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Widefield SIM — Apotome evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 4 OF 8

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 151 Slices (4.5 μm); Channels: 2; Scaling (Per Pixel): 0.059 μm X 0.059 μm X 0.030 μm ; Image size (Pixels): 654 X 1110; Image Size (Pixels): 654 X 1110; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.27 μs ; Frame Time: 4.10 s. Illumination: Laser Wavelength: 639 nm @0.09%; 405 nm @0.2%. Optical/scan: Pinhole: 1.00 AU / 66 μm ; 1.00 AU / 42 μm ; Scan Zoom: 1.8; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: 8.0 (3D, Auto); 6.6 (3D, Auto). Structured source metadata values: 45.

VISIBLE OBSERVATION

A preserved presentation image is available for Confocal. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy and antibody-validation guidance that presentation evidence must remain bounded by modality, controls and source documentation.

INTERPRETIVE LIMIT

Optical sectioning and source-bounded 3D presentation do not establish binding, mechanism, or population-level effect.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Confocal evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 5 OF 8

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 171 Slices (5.1 μm); Channels: 2; Scaling (Per Pixel): 35.28 nm X 35.28 nm X 30.00 nm; Image size (Pixels): 1503 X 1503; 481 X 274; Image Size (Pixels): 1503 X 1503; 481 X 274; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.69 μs ; Frame Time: 7.51 s. Illumination: Laser Wavelength: 639nm @2.00%; 405 nm @0.2%. Optical/scan: Pinhole: 5.00 AU / 328 μm ; 5.00 AU / 215 μm ; Scan Zoom: 2.5; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: SuperResolution 8.6 (3D, Auto); SuperResolution 7.5 (3D, Auto). Structured source metadata values: 50.

VISIBLE OBSERVATION

A preserved presentation image is available for Airyscan. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy guidance that advanced-resolution outputs remain dependent on acquisition, reconstruction or localization quality control.

INTERPRETIVE LIMIT

Displayed feature separation remains reconstruction dependent; no unsupported numerical resolution or molecular architecture claim is permitted.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Airyscan evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 6 OF 8

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 114 Slices (3.39 μm); Channels: 3; Scaling (Per Pixel): 13.20 nm X 13.03 nm X 30.00 nm; 0.013 μm X 0.013 μm X 0.030 μm ; Image size (Pixels): 2860 X 4951; 968 X 898; Image Size (Pixels): 2860 X 4951; 968 X 898; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820; AxioCam 820 #2. Timing: Exposure Time: 150 ms; 120 ms; Frame Time: 1.98 s; 1.59 s. Illumination: Laser Wavelength: 640 nm @4.00%; 488 nm @1.50%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 66.

VISIBLE OBSERVATION

A preserved presentation image is available for SIM. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy guidance that advanced-resolution outputs remain dependent on acquisition, reconstruction or localization quality control.

INTERPRETIVE LIMIT

Reconstruction-dependent structural separation requires native reconstruction QC before numerical resolution or molecular dimensions can be claimed.

CLAIM CEILING

This tier supports a source-bounded statement that the historical SIM evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 7 OF 8

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 114 Slices (3.39 μ m); Channels: 3; Scaling (Per Pixel): 13.20 nm X 13.20 nm X 30.00 nm; 13.20 nm X 13.20 nm X 30.00 μ m; Image size (Pixels): 2860 X 4951; 1501 X 1638; Image Size (Pixels): 2860 X 4951; 1501 X 1638; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820; AxioCam 820 #2. Timing: Exposure Time: 150 ms; 120 ms; Frame Time: 1.98 s; 1.59 s. Illumination: Laser Wavelength: 640 nm @4.00%; 488 nm @1.50%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM²; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 73.

VISIBLE OBSERVATION

A preserved presentation image is available for SIM². BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as SIM², documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy guidance that advanced-resolution outputs remain dependent on acquisition, reconstruction or localization quality control.

INTERPRETIVE LIMIT

Further reconstruction-dependent segmentation does not establish molecular dimensions, structure identity, or mechanism without independent support.

CLAIM CEILING

This tier supports a source-bounded statement that the historical SIM² evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 8 OF 8

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Bruker Vutara VXL. Objective: Olympus 60X/1.30 silicon oil objective. Platform: Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:.

Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20ms Selected; Super Resolution Camera Exposure Time: 20ms; Widefield Camera Exposure Time: 100ms. Illumination: Photoactivation/Imaging Laser: 0% Selected.

Structured source metadata values: 71.

VISIBLE OBSERVATION

A preserved presentation image is available for Single-molecule STORM. BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as Single-molecule STORM, documents platform context as Bruker Vutara VXL, and records detected dye/channel descriptors as 647.

LITERATURE CORRELATION

Consistent with peer-reviewed microscopy guidance that advanced-resolution outputs remain dependent on acquisition, reconstruction or localization quality control.

INTERPRETIVE LIMIT

Without localization tables, uncertainty, blinking, drift, and independent cluster evidence, the presentation cannot establish molecule count, cluster truth, exact precision, or molecular architecture.

CLAIM CEILING

This tier supports a source-bounded statement that the historical Single-molecule STORM evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

CROSS-MODALITY SYNTHESIS

The preserved DC-000013 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

Different acquisitions are not treated as a quantitative intensity ladder. Any continuity statement remains qualitative and source bounded.

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

BENNETT uses independently verified primary scientific sources to test whether the interpretation is scientifically consistent and properly bounded. Literature correlation does not retroactively validate the Identifyn dataset or replace scientist review.

- Antibody and microscopy evidence require orthogonal controls and modality-appropriate interpretation; presentation images alone do not establish analytical specificity.
- Advanced-resolution presentation depends on acquisition, reconstruction or localization quality control before numerical resolution, molecular dimensions or cluster identity can be claimed.

REFERENCES

1. Uhlen M, et al. A proposal for validation of antibodies. Nat Methods. 2016;13:823-827. doi:10.1038/nmeth.3995.
2. Gustafsson MGL. Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. J Microsc. 2000;198:82-87. doi:10.1046/j.1365-2818.2000.00710.x.
3. Sage D, et al. Quantitative evaluation of software packages for single-molecule localization microscopy. Nat Methods. 2015;12:717-724. doi:10.1038/nmeth.3442.

RELEASE BOUNDARY

This assessment remains a draft pending scientist review. No publication is authorized. Any future native-file analysis must enter BENNETT as a new evidence snapshot with routed engine authority, native-data QA, methods, limitations, and an independently reviewed claim ceiling.

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

Historical Identifyn source material was subjected to BENNETT retrospective QA. Original source state and correction history remain preserved in provenance.

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.28 nm X 35.28 nm X 30.00 nm -> 0.03528 μ m X 0.03528 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1503 X 1503; Image Size (Scaled)=53.03 μ m X 53.03 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 13.20 nm X 13.03 nm X 30.00 nm -> 1.00000 μ m X 1.00000 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=2860 X 4951; Image Size (Scaled)=2860 μ m X 4951 μ m; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 13.20 nm X 13.20 nm X 30.00 nm -> 0.01320 μ m X 0.01320 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=2860 X 4951; Image Size (Scaled)=37.74 μ m X 65.33 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.04%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 13.20 nm X 13.20 nm X 30.00 μ m -> 0.01320 μ m X 0.01320 μ m X 0.03000 μ m. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=2860 X 4951; Image Size (Scaled)=37.74 μ m X 65.33 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.04%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

Cross-tier / treatment concentration: 200 μ M; 2 μ M -> Etoposide = 2 μ M. The governing scientist identified every 200 μ M occurrence as a technician transcription error. The canonical historical concentration is 2 μ M. Supporting evidence: Scientist-authorized correction supplied for the Identifyn historical archive; original technician text remains preserved unchanged. Authority: Dr. Brian T. Bennett. Preservation: Original source text and hashes remain unchanged; only the derivative canonical field is corrected.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["24 hours"], "Widefield": ["24 hours"], "Widefield SIM — Apotome": ["24 hours"], "Confocal": ["24 hours"], "Airyscan": ["24 hours"], "SIM": ["24 hours"], "SIM²": ["24 hours"], "Single-molecule STORM": ["6 hours"]} -> Preserved as modality-specific historical duration values. Genuinely different treatment durations are present across evidence stages, and no source or scientist correction resolves them. Supporting evidence: Only duration values from explicit etoposide-treatment sentences were classified; concentration, wavelength, resolution and pixel-size values were excluded.

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

BENNETT has completed the bounded retrospective assessment.
The following pages switch ownership to the preserved Identifyn record.



IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

Images, source methods, acquisition settings and provenance follow in the locked
Stepdown order. Scientific wording is preserved; missing modalities are not invented.
No separate control record is present in the preserved product record.

PRODUCT-LEVEL STEPDOWN MAP

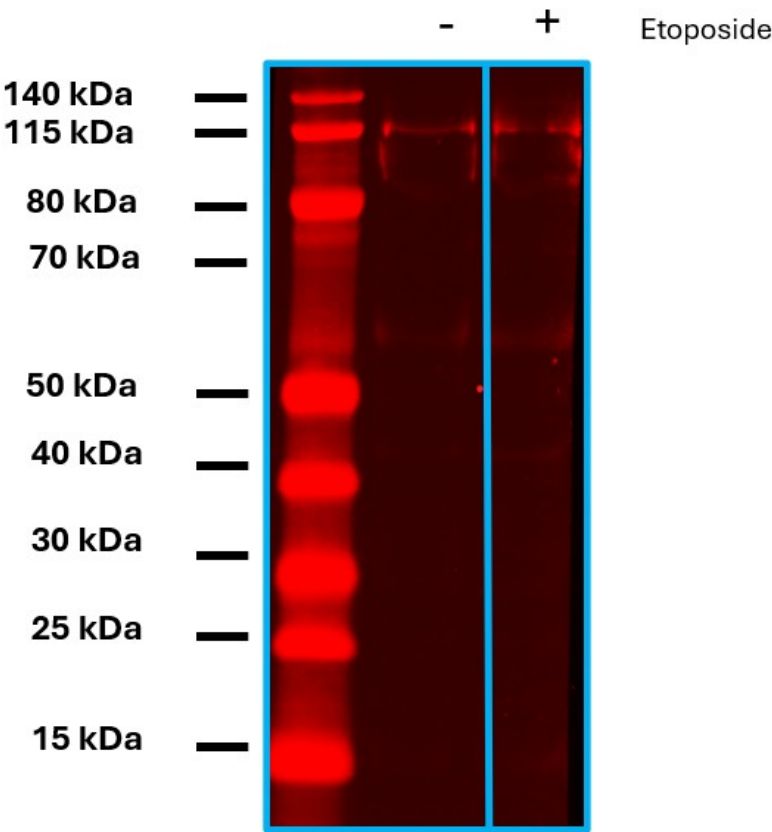
This dossier preserves the complete available evidence progression for DC-000013. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM ²	ZEISS Elyra	PRESERVED
8	Single-molecule STORM	Bruker Vutara VXL	PRESERVED

INTERPRETIVE BOUNDARY

Ordering evidence by resolution tier does not make the modalities interchangeable. Each stage retains its acquisition environment and scientific limitations. This pilot organizes the record; it does not synthesize new biological claims.

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HEK293
DETECTED DYES	647
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	632nm
Emission Filter	676±37nm
Detector	PMT
Intensity	2
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	K. Small

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M)
Cat ID# - DC 000013

Expected Molecular Weight: 116 kDa

HEK293 cells were damaged with 200 µM etoposide for 24 hours. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer, and then centrifuged to pellet down the cell debris. The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80°C for 10 min, and subjected to electrophoresis using NuPAGE™ 4 to 12%, Bis-Tris, 1.0-1.5 mm, Mini Protein Gels in a Mini Gel Tank. The Thermo Fisher's PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, was used as a molecular weight marker.

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit, PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour and washed 5X with PBS. The blocked membrane was incubated with Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M) for 2 hours, followed by 5X PBS washes. The membrane was dried for 10 minutes at room temperature before imaging.

Control Data - Primary Unconjugated Antibody

Scanner = Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)

Scan Settings

Detection mode = Fluorescence

Laser = 632nm

Emission Filter = 676±37nm

Detector = PMT

Intensity = 2

Pixel size = 100 µm

Scan Speed = High

Quality = High

Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: K. Small

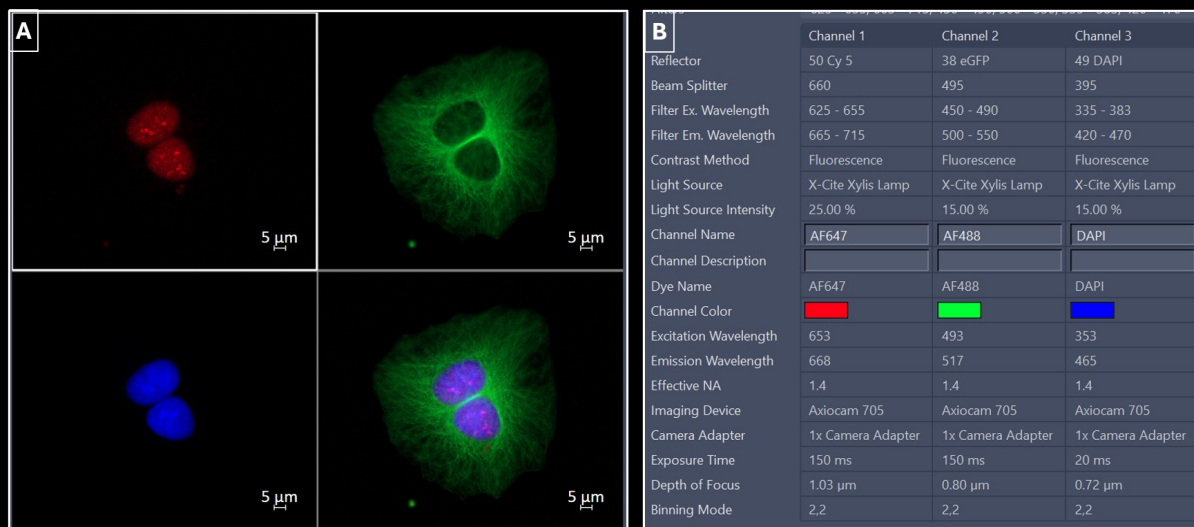
Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE

Catalog	DC-000013
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HEK293
Image SHA-256	3198E2ADE95D5F5BB55FF497B9F90E4FD69D67846400F597C38DA9D77E31A623
Method PDF SHA-256	C8A395454537F47B543BEAA0D8265D6E6984E2BD3D9BF84FCAE4DCD57584BA85

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Panel A - Split View illustrates the signal and localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn SRM Alexa Fluor 647, IgG, Direct Conjugate. Upper left, PARP1 647 direct conjugate (red), upper right, alpha tubulin primary antibody labeled with Identifyn SRM Alexa Fluor 488, while the lower left displays DAPI (blue). The lower right presents a merged image of the three channels.

Panel B - Displays the settings used for image acquisition within Zeiss Zen software. The Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn SRM Alexa Fluor 647, IgG, Direct Conjugate was captured using 25% LED for 150 ms, while the Identifyn SRM Alexa Fluor 488 was captured with 15% LED for 50 ms. The image settings indicate that the 647 direct conjugate is bright and easily captured with LED, as is the Identifyn 488.

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	37

STRUCTURED ACQUISITION METADATA / WIDEFIELD

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	3
Scaling (Per Pixel)	0.110 μ m X 0.110 μ m
Image size (Pixels)	1216 X 1028
Image Size (Scaled)	133.18 μ m X 112.59 μ m
Bit Depth	14 Bit
Image Center Position	X: 0.00 μ m, Y: 0.00 μ m
ROI Center Offset	X: 0.00 μ m, Y: 0.00 μ m
Reflector	50 Cy 5 38 eGFP 49 DAPI
Beam Splitter	660 495 395
Filter Excitation Wavelength	625 - 655 450 - 490 335 - 383
Filter Emission Wavelength	665 - 715 500 - 550 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25.00% X-Cite Xylis Lamp Intensity @15.00%
Exposure Time	150 ms 20 ms
Excitation Wavelength	653 493 353
Emission Wavelength	668 517 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.03 μ m 0.80 μ m 0.72 μ m
Binning Mode	2,2

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M)
Cat ID# - DC 000013

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000015

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD⁺; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process. PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D)

Channels = 3

Scaling (Per Pixel) = 0.110 µm X 0.110 µm

Image size (Pixels) = 1216 X 1028

Image Size (Scaled) = 133.18 µm X 112.59 µm

Bit Depth = 14 Bit

Image Center Position = X: 0.00 µm, Y: 0.00 µm

ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = 50 Cy 5
 Beam Splitter = 660
 Filter Excitation Wavelength = 625 - 655
 Filter Emission Wavelength = 665 - 715
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @25.00%
 Exposure Time = 150 ms
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 1.03 μ m
 Binning Mode = 2,2

488 -

Reflector = 38 eGFP
 Beam Splitter = 495
 Filter Excitation Wavelength = 450 - 490
 Filter Emission Wavelength = 500 - 550
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @15.00%
 Exposure Time = 150 ms
 Excitation Wavelength = 493
 Emission Wavelength = 517
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.80 μ m
 Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
 Beam Splitter = 395
 Filter Excitation Wavelength = 335 - 383
 Filter Emission Wavelength = 420 - 470
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @15.00%
 Exposure Time = 20 ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1X
 Depth of Focus = 0.72 μ m
 Binning Mode = 2,2

Split View - Panel A

The top-left panel shows the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M) after DNA damage, the top-right panel features Alpha Tubulin visualized with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L), and the bottom-left panel features DAPI nuclear staining. The bottom-right panel is the merged image of all channels.

Summary

Panel A - Split View illustrates the signal and localization of Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG, Direct Conjugate. Upper left, PARP1 647 direct conjugate (red), upper right, alpha tubulin primary antibody labeled with Identifyn® SRM Alexa Fluor™ 488, while the lower left displays DAPI (blue). The lower right presents a merged image of the three channels.

Panel B - Displays the settings used for image acquisition within Zeiss Zen software. The Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG, Direct Conjugate was captured using 25% LED for 150 ms. In comparison, the Identifyn® SRM Alexa Fluor™ 488 was captured with 15% LED for 50 ms. The image settings indicate that the 647 direct conjugate is bright and easily captured with LED, as is the Identifyn® 488.

Image Correction

None

Image by E.F. Simon

Image Analysis by B.T. Bennett

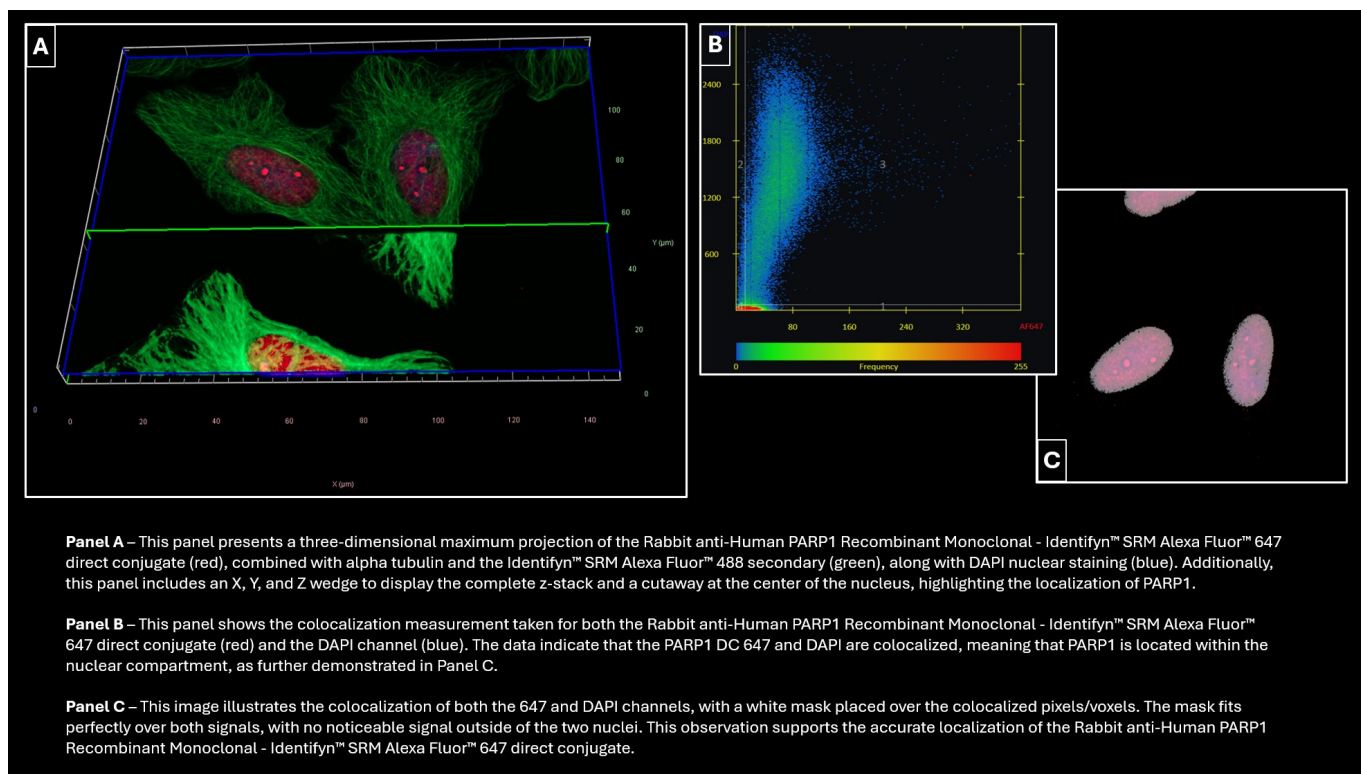
Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE

Catalog	DC-000013
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, AxioCam 705, and X-Cite Xylys Lamp, was used with the following parameters for each dye:
Structured source metadata values	37
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	7F7E22FD6A3498EE6D08920AFDFAAEDA3C2FE3AD601AE0AE0B864BE9E7C4B34E
Method PDF SHA-256	C8EB09EA319AB16F31CBCDF0D2650C3D1F589E99CA5C9293B6AC115D71CE0AD3

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	46

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Z-Stack	92 Slices (9.1 μm)
Channels	3
Scaling (Per Pixel)	0.143 μm X 0.143 μm X 0.100 μm
Image Size (Pixels)	1037 X 808
Image Size (Scaled)	148.14 μm X 115.43 μm
Bit Depth	14 Bit
Image Center Position	X: 56.22 mm, Y: 49.95 mm
Stage Position	= X: 56.22 mm, Y: 49.95 mm
ROI Center Offset	X: 1.14 μm , Y: 0.00 μm
Reflector	50 Cy 5 38 HE eGFP 49 DAPI
Beam Splitter	660 495 395
Filter Excitation Wavelength	625 - 655 450 - 490 335 - 383
Filter Emission Wavelength	665 - 715 500 - 550 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @15.00% X-Cite Xylis Lamp Intensity @10.00%
Exposure Time	300 ms 100 ms 20 ms
Excitation Wavelength	653 493 353
Emission Wavelength	668 517 465
Effective NA	1.25
Imaging Device	807 mono
Camera Adapter	1X
Depth of Focus	1.30 μm 1.00 μm 0.90 μm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels.
Background	Black
Light	Brightness 100%, Tone Mapping Enabled
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M)
Cat ID# - DC 000013

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L)
Cat ID# - SA 000017

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD⁺; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process. PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')₂ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, AxioCam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 92 Slices (9.1 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 1037 X 808
Image Size (Scaled) = 148.14 µm X 115.43 µm
Bit Depth = 14 Bit
Image Center Position = X: 56.22 mm, Y: 49.95 mm
Stage Position = X: 56.22 mm, Y: 49.95 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

647-

Reflector = 50 Cy 5
Beam Splitter = 660
Filter Excitation Wavelength = 625 - 655
Filter Emission Wavelength = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15.00%
Exposure Time = 300 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.30 µm
Binning Mode = 2,2

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @10.00%
Exposure Time = 100 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = 807 mono
Camera Adapter = 1X
Depth of Focus = 1.00 µm
Binning Mode = 2,2

DAPI -

Reflector = 49 DAPI
 Beam Splitter = 395
 Filter Excitation Wavelength = 335 - 383
 Filter Emission Wavelength = 420 - 470
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @10.00%
 Exposure Time = 20 ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.25
 Imaging Device = 807 mono
 Camera Adapter = 1X
 Depth of Focus = 0.90 μ m
 Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected.

Normalization, Phase Correction, Fourier Filter, and Deconvolution were NOT used.

3D Image Processing - Panel A

3D Maximum Projection = Precise
 Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels.
 Background = Black
 Light = Brightness 100%, Tone Mapping Enabled
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

X-Y and X-Z Wedge selected

Colocalization View - Panel B

Horizontal Axis - AF647, Threshold 13, Range Auto
 Vertical Axis - DAPI, Threshold 61, Range Auto

Summary

Panel A - This panel presents a three-dimensional maximum projection of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 direct conjugate (red), combined with alpha tubulin and the Identifyn® SRM Alexa Fluor™ 488 secondary (green), along with DAPI nuclear staining (blue). Additionally, this panel includes an X, Y, and Z wedge to display the complete z-stack and a cutaway at the center of the nucleus, highlighting the localization of PARP1.

Panel B - This panel shows the colocalization measurement taken for both the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 direct conjugate (red) and the DAPI channel (blue) The data indicate that the PARP1 DC 647 and DAPI are colocalized, meaning that PARP1 is located within the nuclear compartment, as further demonstrated in Panel C.

Panel C - This image illustrates the colocalization of both the 647 and DAPI channels, with a white mask placed over the colocalized pixels/voxels. The mask fits perfectly over both signals, with no noticeable signal outside of the two

nuclei. This observation supports the accurate localization of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 direct conjugate.

Image Correction

None

Image by E.F. Simon

Image Analysis by B.T. Bennett

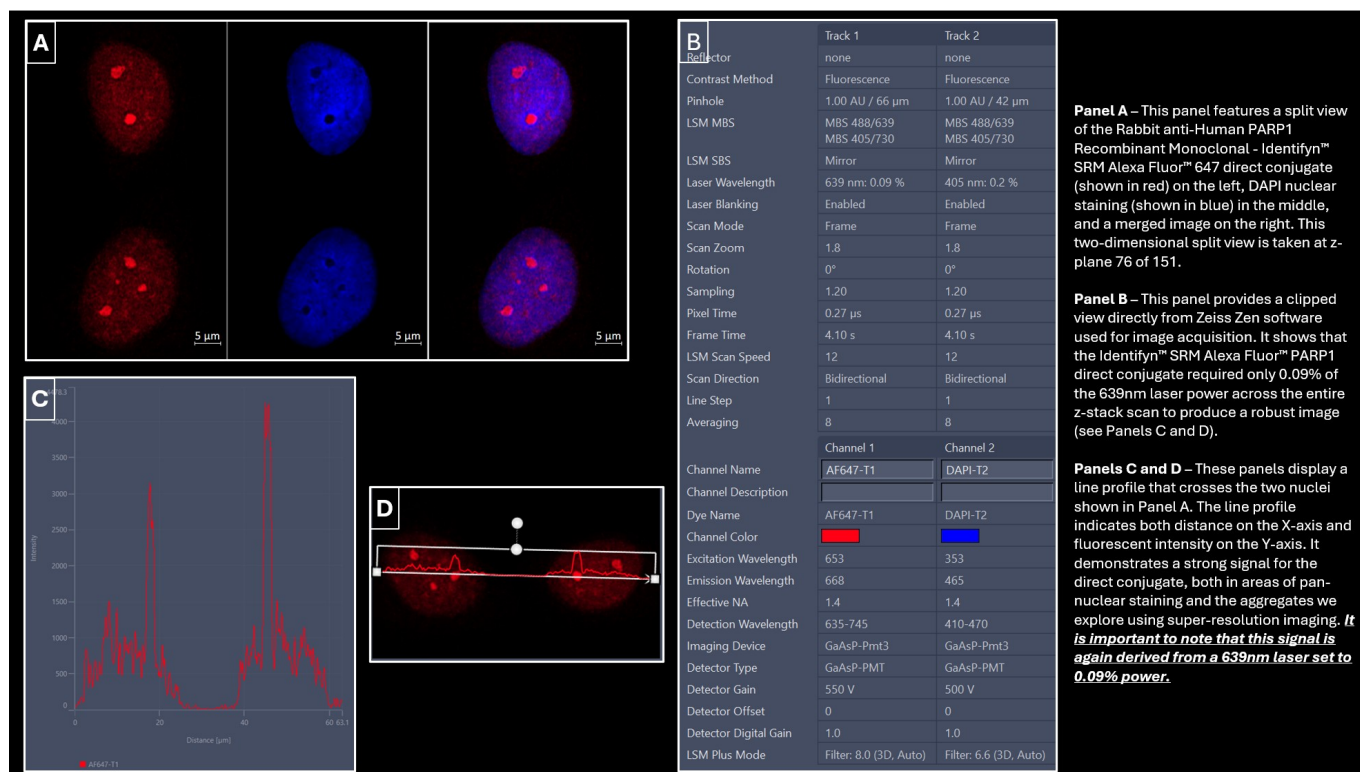
Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE

Catalog	DC-000013
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	AB96AFB5816780090E0135EE5D216FCD2FF0C3D7A50B59B5F48AA5EFE9FECF49
Method PDF SHA-256	BD6B40ABE8D0880E81548B6AD6754CF7ABF3FEB3F2A61B5F69F9D8099BE47C0

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	45

STRUCTURED ACQUISITION METADATA / CONFOCAL

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	151 Slices (4.5 μm)
Channels	2
Scaling (Per Pixel)	0.059 μm X 0.059 μm X 0.030 μm
Image Size (Pixels)	654 X 1110
Image Size (Scaled)	38.47 μm X 65.30 μm
Bit Depth	16 Bit
Image Center Position	X: 102.14 mm, Y: 57.27 mm
Stage Position	X: 102.14 mm, Y: 57.27 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 66 μm 1.00 AU / 42 μm
LSM MBS	MBS 488/639, 405/730
LSM SBS	Mirror
Laser Wavelength	639 nm @0.09% 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.8
Rotation	0°
Sampling	1.20
Pixel Time	0.27 μs
Frame Time	4.10 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	653 353
Emission Wavelength	668 465
Effective NA	1.4
Detection Wavelength	635-745 410-470
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	550 V 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	8.0 (3D, Auto) 6.6 (3D, Auto)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 63.1), Y (Min 0 and Max 4478)

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M)
Cat ID# - DC 000013

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD⁺; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process. PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Cells were washed 5X in PBS and mounted using ProLong Diamond Antifade Mountant with DAPI (cat#P36962).

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D) - Panels A, C, & D

Z-Stack = 151 Slices (4.5 µm)

Channels = 2

Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm

Image Size (Pixels) = 654 X 1110

Image Size (Scaled) = 38.47 µm X 65.30 µm

Bit Depth = 16 Bit

Image Center Position = X: 102.14 mm, Y: 57.27 mm

Stage Position = X: 102.14 mm, Y: 57.27 mm

ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 66 μ m
 LSM MBS = MBS 488/639, 405/730
 LSM SBS = Mirror
 Laser Wavelength = 639 nm @0.09%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.8
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.27 μ s
 Frame Time = 4.10 s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.4
 Detection Wavelength = 635-745
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 550 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 8.0 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 42 µm
 LSM MBS = MBS 488/639, 405/730
 LSM SBS = Mirror
 Laser Wavelength = 405 nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.8
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.27 µs
 Frame Time = 4.10 s
 LSM Scan Speed = 12
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 410-470
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 500 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = 6.6 (3D, Auto)

Split View - Panel A

The left panel shows the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M) after DNA damage, the middle panel features DAPI nuclear staining, and the right panel is the merged image of all channels.

Profile View Display - Panels C & D

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1
 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 63.1), Y (Min 0 and Max 4478)

Summary

Panel A - This panel features a split view of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 direct conjugate (shown in red) on the left, DAPI nuclear staining (shown in blue) in the middle, and a merged image on the right. This two-dimensional split view is taken at z-plane 76 of 151.

Panel B - This panel provides a clipped view directly from Zeiss Zen software used for image acquisition. It shows that the Identifyn® SRM Alexa Fluor™ PARP1 direct conjugate required only 0.09% of the 639nm laser power across the entire z-stack scan to produce a robust image (see Panels C and D).

Panels C and D - These panels display a line profile that crosses the two nuclei shown in Panel A. The line profile indicates both distance on the X-axis and fluorescent intensity on the Y-axis. It demonstrates a strong signal for the

direct conjugate, both in areas of pan-nuclear staining and the aggregates we explore using super-resolution imaging. It is important to note that this signal is again derived from a 639nm laser set to 0.09% power.

Image Corrections

None

Image by J.K. Bennett

Image Analysis by B.T. Bennett

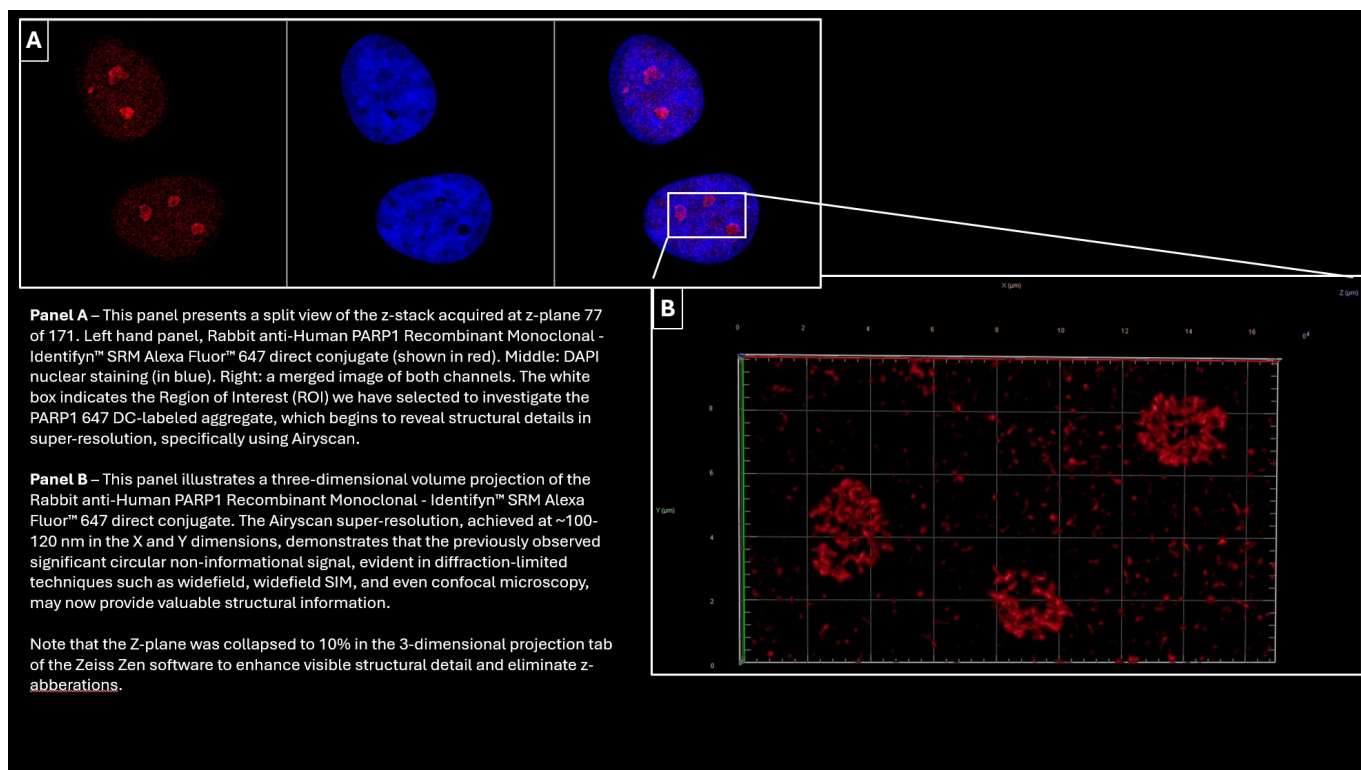
Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE

Catalog	DC-000013
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	45
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	E4C49CE7EA37A728BBB41DC96F940044EB4519AB8DC34C1F8FF250F6DFF01CE2
Method PDF SHA-256	D9D49A386CEA23AA325AD91F06F2276C19858A0AB991BAE226B94632FBA410DA

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	50

STRUCTURED ACQUISITION METADATA / AIRYSCAN

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	171 Slices (5.1 μm)
Channels	2
Scaling (Per Pixel)	0.03528 μm X 0.03528 μm X 0.03000 μm
Image Size (Pixels)	1503 X 1503 481 X 274
Image Size (Scaled)	53.03 μm X 53.03 μm 16.97 μm X 9.67 μm
Bit Depth	16 Bit
Image Center Position	X: 72.75 mm, Y: 49.55 mm
Stage Position	X: 72.75 mm, Y: 49.55 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 328 μm 5.00 AU / 215 μm
LSM MBS	MBS 488/639, 405/730
LSM SBS	SBS LP 640 SBS SP 505
Laser Wavelength	639nm @2.00% 405 nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.5
Rotation	0°
Sampling	2.00
Pixel Time	0.69 μs
Frame Time	7.51 s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	653 353
Emission Wavelength	668 465
Effective NA	1.4
Detection Wavelength	643-735 350-499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	SuperResolution 8.6 (3D, Auto) SuperResolution 7.5 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 4%, Ramp 69%, Maximum 100%
Background	Black
Light	Brightness 95%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M)
Cat ID# - DC 000013

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD⁺; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process. PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Cells were washed 5X in PBS and mounted using ProLong Diamond Antifade Mountant with DAPI (cat#P36962).

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 171 Slices (5.1 µm)

Channels = 2

Scaling (Per Pixel) = 35.28 nm X 35.28 nm X 30.00 nm

Image Size (Pixels) = 1503 X 1503

Image Size (Scaled) = 53.03 µm X 53.03 µm

Bit Depth = 16 Bit

Image Center Position = X: 72.75 mm, Y: 49.55 mm

Stage Position = X: 72.75 mm, Y: 49.55 mm

ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B

Z-Stack = 171 Slices (5.1 µm)

Channels = 2

Scaling (Per Pixel) = 35.28 nm X 35.28 nm X 30.00 nm

Image Size (Pixels) = 481 X 274

Image Size (Scaled) = 16.97 µm X 9.67 µm

Bit Depth = 16 Bit

Image Center Position = X: 72.75 mm, Y: 49.55 mm

Stage Position = X: 72.75 mm, Y: 49.55 mm

ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None

Contrast Method = Fluorescence

Pinhole = 5.00 AU / 328 µm

LSM MBS = MBS 488/639, 405/730

LSM SBS = SBS LP 640

Laser Wavelength = 639nm @2.00%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 2.5

Rotation = 0°

Sampling = 2.00

Pixel Time = 0.69 µs

Frame Time = 7.51 s

LSM Scan Speed = 7

Scan Direction = Bidirectional

Line Step = 1

Averaging = 4

Excitation Wavelength = 653

Emission Wavelength = 668

Effective NA = 1.4

Detection Wavelength = 643-735

Imaging Device = Airyscan

Detector Type = GaAsP-PMT

Detector Gain = 800 V

Detector Offset = 0

Detector Digital Gain = 1.0

Airyscan Mode = SuperResolution 8.6 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 215 µm
 LSM MBS = MBS 488/639, 405/730
 LSM SBS = SBS SP 505
 Laser Wavelength = 405 nm @0.2%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.5
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.69 µs
 Frame Time = 7.51 s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 4
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 350-499
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 800 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 Airyscan Mode = SuperResolution 7.5 (3D, Auto)

Post Processing - AiryScan 3D Processing

Display Mode "SR"
 Super Resolution Auto Filter Selected
 Process 3D Current Image
 Created

Split View - Panel A

The left panel shows the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M) after DNA damage, the middle panel features DAPI nuclear staining, and the right panel is the merged image of all channels.

3D Image Processing - Panel B

3D Volume Projection = Precise
 Appearance = Threshold 4%, Ramp 69%, Maximum 100%
 Background = Black
 Light = Brightness 95%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - This panel presents a split view of the z-stack acquired at z-plane 77 of 171. Left-hand panel, Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 direct conjugate (shown in red). Middle: DAPI nuclear staining (in blue). Right: a merged image of both channels. The white box indicates the Region of Interest (ROI) we have selected to investigate the PARP1 647 DC-labeled aggregate, which begins to reveal structural details in super-resolution, specifically using Airyscan.

Panel B - This panel illustrates a three-dimensional volume projection of the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 direct conjugate The Airyscan super-resolution, achieved at ~100-120 nm in the X and Y dimensions, demonstrates that the previously observed significant circular non-informational signal, evident in diffraction-limited techniques such as widefield, widefield SIM, and even confocal microscopy, may now provide valuable structural information.

Note that the Z-plane was collapsed to 10% in the 3-dimensional projection tab of the Zeiss Zen software to enhance visible structural detail and eliminate z-aberrations.

Image Corrections

None

Image by J.K. Bennett

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE

Catalog	DC-000013
Stage	Airyscan
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	B71770C0279EBE2E9ADC112ED9136E8E249DA1C6D459E99334963BB8B175986E
Method PDF SHA-256	B0EDC8E640288B3391108CF30CF92C4835E9BC5AD4546A992F7A853BB7A192D2

[illegible]

EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	66

STRUCTURED ACQUISITION METADATA / SIM

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	114 Slices (3.39 μm)
Channels	3
Scaling (Per Pixel)	1.00000 μm X 1.00000 μm X 0.03000 μm 0.013 μm X 0.013 μm X 0.030 μm
Image Size (Pixels)	2860 X 4951 968 X 898
Image Size (Scaled)	2860 μm X 4951 μm 12.77 μm X 11.85 μm
Bit Depth	16 Bit
Image Center Position	X: 58.82 mm, Y: 38.45 mm
Stage Position	X: 58.82 mm, Y: 38.45 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 488 405
Channel Name	T1-Axiocam 820-SR T2-Axiocam 820-SR T3-Axiocam 820 #2-SR
Excitation Wavelength	640 488 405
Emission Wavelength	729 525 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 Axiocam 820 #2
Camera Adapter	1X
Exposure Time	150 ms 120 ms 50 ms
Depth of Focus	1.13 μm 0.81 μm 0.69 μm
Binning Mode	2,2
Laser Wavelength	640 nm @4.00% 488 nm @1.50% 405 nm @7.00%
Frame Time	1.98 s 1.59 s 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 μm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	N/A
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.6089 (647), 9.9753 (488), 8.5469 (DAPI)
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 3% all channels, Ramp 0% all channels, Maximum 100% all channels Threshold 3% all channels, Ramp 97% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 88%, Enabled Tone Mapping Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M)
Cat ID# - DC 000013

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)
Cat ID# - SA 000019

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD⁺; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process. PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Mitochondria Antibody (MA5-12017), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 114 Slices (3.39 µm)

Channels = 3
Scaling (Per Pixel) = 13.20 nm X 13.03 nm X 30.00 nm
Image Size (Pixels) = 2860 X 4951
Image Size (Scaled) = 2860 µm X 4951 µm
Bit Depth = 16 Bit
Image Center Position = X: 58.82 mm, Y: 38.45 mm
Stage Position = X: 58.82 mm, Y: 38.45 mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panel B

Z-Stack = 114 Slices (3.39 µm)

Channels = 3
Scaling (Per Pixel) = 0.013 µm X 0.013 µm X 0.030 µm
Image Size (Pixels) = 968 X 898
Image Size (Scaled) = 12.77 µm X 11.85 µm
Bit Depth = 16 Bit
Image Center Position = X: 58.82 mm, Y: 38.45 mm
Stage Position = X: 58.82 mm, Y: 38.45 mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820-SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1X
Exposure Time = 150 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @4.00%
Frame Time = 1.98 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 488
 Channel Name = T2-Axiocam 820-SR
 Excitation Wavelength = 488
 Emission Wavelength = 525
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820
 Camera Adapter = 1X
 Exposure Time = 120 ms
 Depth of Focus = 0.81 μm
 Binning Mode = 2,2
 Laser Wavelength = 488 nm @1.50%
 Frame Time = 1.59 s
 Scan Direction = Unidirectional
 Grating = 36.5 μm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T3-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 445
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 50 ms
 Depth of Focus = 0.69 μm
 Binning Mode = 2,2
 Laser Wavelength = 405 nm @7.00%
 Frame Time = 676.00 ms
 Scan Direction = Unidirectional
 Grating = 36.5 μm grating

Post Processing - SIM Processing

Processing Dimension = 3D

Result Sampling = 4

Process Sampling = N/A

Channels = 3

Algorithm = SIM

SIM Settings = Standard

Auto Sharpness = On

Sharpness = 9.6089 (647), 9.9753 (488), 8.5469 (DAPI)

Fitting = Fast Fit

Normalization = Auto

3D Image Processing - Panel A

3D Maximum Projection = Precise

Appearance = Threshold 3% all channels, Ramp 0% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 88%, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel B

3D Volume Projection = Precise

Appearance = Threshold 3% all channels, Ramp 97% all channels, Maximum 100% all channels

Background = Black

Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - This panel presents a 3-dimensional maximum projection of the z-stack acquired Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 direct conjugate (shown in red). Mitochondrial primary antibody counterstained with Identifyn® SRM Alexa Fluor™ 488 secondary (in green), DAPI nuclear staining (in blue).

Panel B - This panel presents a 3-dimensional volume projection of the Region of Interest (ROI) shown in Panel A. Here, we see the structural detail of the PARP1 647 direct conjugate at the aggregate level. We note that validation of the resolution is evident in the mitochondrial imaging, as the structure and center of the mitochondria are apparent, as we have shown to be highly reproducible at SIM resolution.

Panel C - This panel is a clipped view of the acquisition settings for this z-stack; we provide it to enable transparency in our image settings and demonstrate the Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 direct conjugate was captured over 114 z-planes using a 639nm laser at 4% input, providing valuable information regarding the excellent brightness of the Identifyn® conjugation.

Image Corrections

None

Image by P. Raut

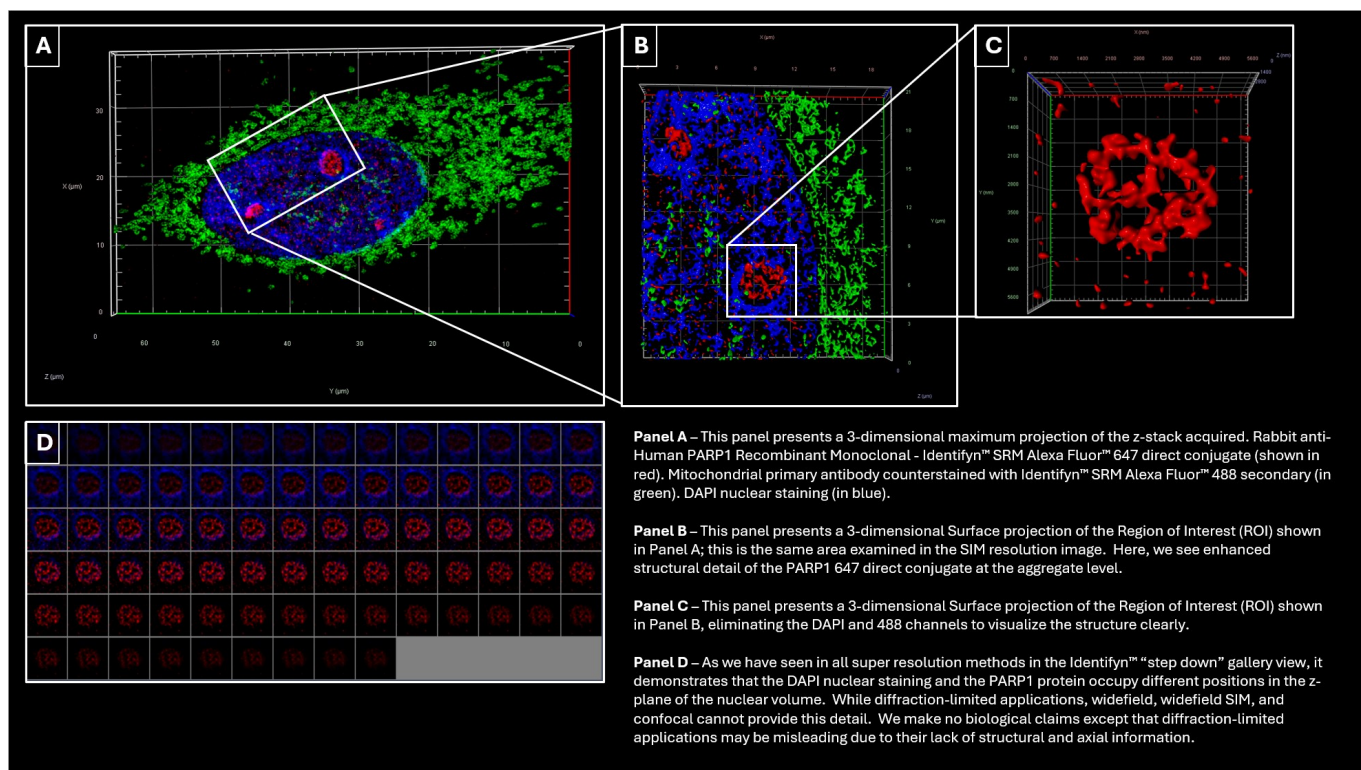
Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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SOURCE PROVENANCE

Catalog	DC-000013
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	66
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	489D70569C5BD7C304BF7842388CD20FF4953744987940D37D22A6373FDE24C8
Method PDF SHA-256	9666BCBDD3643FEE43EF953CBCE111B9BCA0A486A1E94ED35251C40F5ADF6F40

ORIGINAL IMAGE EVIDENCE / SIM²

EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	73

STRUCTURED ACQUISITION METADATA / SIM²

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	114 Slices (3.39 μm)
Channels	3
Scaling (Per Pixel)	0.01320 μm X 0.01320 μm X 0.03000 μm 0.01320 μm X 0.01320 μm X 0.03000 μm
Image Size (Pixels)	2860 X 4951 1501 X 1638 440 X 459
Image Size (Scaled)	37.74 μm X 65.33 μm 19.81 μm X 21.61 μm 5.81 μm X 6.06 μm
Bit Depth	16 Bit
Image Center Position	X: 58.82 mm, Y: 38.45 mm
Stage Position	X: 58.82 mm, Y: 38.45 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm X: 0.00 μm , Y: 0.00 μm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 488 405
Channel Name	T1-Axiocam 820-SR T2-Axiocam 820-SR T3-Axiocam 820 #2-SR
Excitation Wavelength	640 488 405
Emission Wavelength	729 525 445
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820 Axiocam 820 #2
Camera Adapter	1X
Exposure Time	150 ms 120 ms 50 ms
Depth of Focus	1.13 μm 0.81 μm 0.69 μm
Binning Mode	2,2
Laser Wavelength	640 nm @4.00% 488 nm @1.50% 405 nm @7.00%
Frame Time	1.98 s 1.59 s 676.00 ms
Scan Direction	Unidirectional
Grating	36.5 μm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	4
Algorithm	SIM ²
SIM ² Settings	Standard
Input SNR	Low
Regularization	None
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Maximum Projection	Precise
Appearance	Threshold 3% all channels, Ramp 0% all channels, Maximum 100% all channels Threshold 20%, Ambient Light 0%, Specular Light 100%, Shininess 30% Threshold 20%, Ambient Light 0%, Specular Light 69%, Shininess 30%
Background	Black
Light	Brightness 88%, Enabled Tone Mapping Azimuth 80°, Elongation -130°, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Surface Projection	Precise

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M)
Cat ID# - DC 000013

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L)
Cat ID# - SA 000019

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD⁺; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process. PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM Etoposide-spiked complete growth media for 24 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS.

The second primary antibody, Mitochondria Antibody (MA5-12017), was then incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, F(ab')₂ (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Control Data - Primary Unconjugated Antibody

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A

Z-Stack = 114 Slices (3.39 µm)

Channels = 3

Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm

Image Size (Pixels) = 2860 X 4951

Image Size (Scaled) = 37.74 µm X 65.33 µm

Bit Depth = 16 Bit

Image Center Position = X: 58.82 mm, Y: 38.45 mm

Stage Position = X: 58.82 mm, Y: 38.45 mm

ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panel B

Z-Stack = 114 Slices (3.39 µm)

Channels = 3

Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 nm

Image Size (Pixels) = 1501 X 1638

Image Size (Scaled) = 19.81 µm X 21.61 µm

Bit Depth = 16 Bit

Image Center Position = X: 58.82 mm, Y: 38.45 mm

Stage Position = X: 58.82 mm, Y: 38.45 mm

ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - Panel C

Z-Stack = 114 Slices (3.39 µm)

Channels = 3

Scaling (Per Pixel) = 13.20 nm X 13.20 nm X 30.00 µm

Image Size (Pixels) = 440 X 459

Image Size (Scaled) = 5.81 µm X 6.06 µm

Bit Depth = 16 Bit

Image Center Position = X: 58.82 mm, Y: 38.45 mm

Stage Position = X: 58.82 mm, Y: 38.45 mm

ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 640
 Channel Name = T1-Axiocam 820-SR
 Excitation Wavelength = 640
 Emission Wavelength = 729
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820
 Camera Adapter = 1X
 Exposure Time = 150 ms
 Depth of Focus = 1.13 μm
 Binning Mode = 2,2
 Laser Wavelength = 640 nm @4.00%
 Frame Time = 1.98 s
 Scan Direction = Unidirectional
 Grating = 36.5 μm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 488
 Channel Name = T2-Axiocam 820-SR
 Excitation Wavelength = 488
 Emission Wavelength = 525
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820
 Camera Adapter = 1X
 Exposure Time = 120 ms
 Depth of Focus = 0.81 μm
 Binning Mode = 2,2
 Laser Wavelength = 488 nm @1.50%
 Frame Time = 1.59 s
 Scan Direction = Unidirectional
 Grating = 36.5 μm grating

DAPI -

Reflectors = SR HE LBF 405/488/561/642
 Beam Splitter = 405, 488, 561, 642
 Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
 Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T3-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 445
 Effective NA = 1.4
 Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 50 ms
 Depth of Focus = 0.69 μ m
 Binning Mode = 2,2
 Laser Wavelength = 405 nm @7.00%
 Frame Time = 676.00 ms
 Scan Direction = Unidirectional
 Grating = 36.5 μ m grating

Post Processing - SIM² Processing

Processing Dimension = 3D
 Result Sampling = 4
 Process Sampling = 4
 Channels = 3
 Algorithm = SIM²
 SIM² Settings = Standard
 Input SNR = Low
 Regularization = None
 Regularization Weight = 0.0000
 Iterations = 3
 Filter = No Filter
 Fitting = Fast Fit
 Normalization = Auto

3D Image Processing - Panel A

3D Maximum Projection = Precise
 Appearance = Threshold 3% all channels, Ramp 0% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 88%, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel B

3D Surface Projection = Precise

Appearance = Threshold 20%, Ambient Light 0%, Specular Light 100%, Shininess 30%

Background = Black

Light = Azimuth 80°, Elongation -130°, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

3D Surface Projection = Precise

Appearance = Threshold 20%, Ambient Light 0%, Specular Light 69%, Shininess 30%

Background = Black

Light = Azimuth 80°, Elongation -130°, Enabled Tone Mapping

Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Summary

Panel A - This panel presents a 3-dimensional maximum projection of the z-stack acquired Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 direct conjugate (shown in red), Mitochondrial primary antibody counterstained with Identifyn® SRM Alexa Fluor™ 488 secondary (in green). DAPI nuclear staining (in blue).

Panel B - This panel presents a 3-dimensional Surface projection of the Region of Interest (ROI) shown in Panel A; this is the same area examined in the SIM resolution image. Here, we see enhanced structural detail of the PARP1 647 direct conjugate at the aggregate level.

Panel C - This panel presents a 3-dimensional Surface projection of the Region of Interest (ROI) shown in Panel B, eliminating the DAPI and 488 channels to visualize the structure.

Panel D - As we have seen in all super resolution methods in the Identifyn® "step down" gallery view, it demonstrates that the DAPI nuclear staining and the PARP1 protein occupy different positions in the z-plane of the nuclear volume. While diffraction-limited applications, widefield, widefield SIM, and confocal cannot provide this detail. We make no biological claims except that diffraction-limited applications may be misleading due to their lack of structural and axial information.

Image Corrections

None

Image by P. Raut

Image Analysis by B.T. Bennett

Data Presentation by P.D. Amistoso

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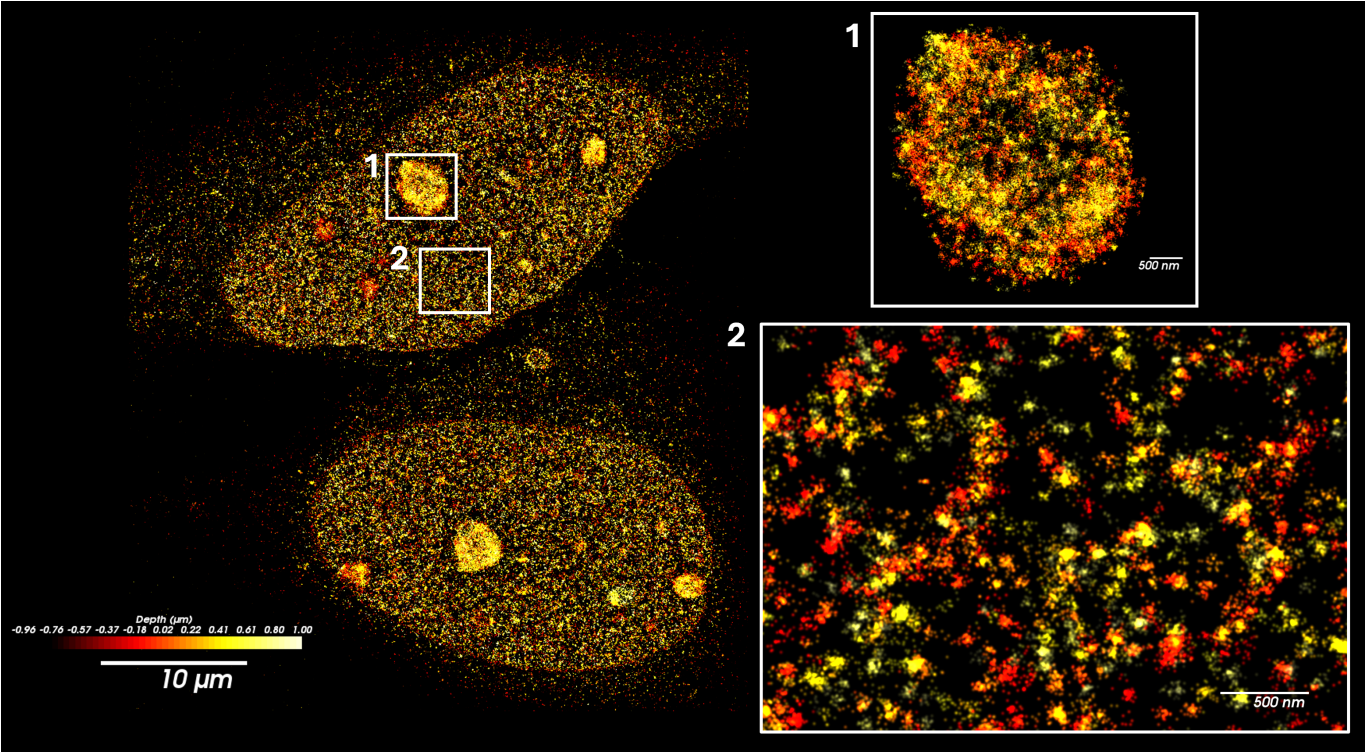
SOURCE PROVENANCE

Catalog	DC-000013
Stage	SIM ²

SOURCE PROVENANCE

Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/ 7 using Plan-Apochromat 63X/1.40 oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	73
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	88FBC7FBDF1A591D238A66A5FDAFBD8A57F8E02742A28F9B85146B222FF4AE2F
Method PDF SHA-256	4EB484A3A86ED62C5CC0BB1A0D2C09957817BDD88397FDA3BE89E6D1C2CE7537

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	71

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

The values below were deterministically extracted from the preserved Identifyn method PDF text. They remain source-documented metadata, not new BENNETT measurements.

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Objective	Olympus 60X/1.30 silicon oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	635nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Course Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100ms 20ms
Super Resolution Camera Exposure Time	20ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200µm
Capture Mode	Live
Piezo Current	181.0 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 640nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control (640 nm (640 nm Dichroic))	Not Selected
Widefield camera Exposure Time	100ms
Widefield Laser	"Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected.
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50µm is selected
Piezo Record	Begin: 180; End: 180.0
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 20000
Z Positions	1 using Steps: 0.2µm (200nm)
Cycles	1
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	10%
Cutout Width	16px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 7
Sizing Mode	Constant
Particle size	20nm
Color by	Constant
Color Table	Single
Opacity Mode	Constant; Opacity 1: 0.8
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	6 Intervals

FIELD	SOURCE-DOCUMENTED VALUE
Pixel Size	30nm
Smoothing	8.00
PSF Z offset	-600 to +600
Radial precision	30
Axial precision	70

SOURCE METHOD

The text below is carried from the preserved Identifyn source record. Typography and pagination have been normalized for this preservation dossier; scientific wording has not been editorially rewritten.

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M)
Cat ID# - DC 000013

PARP1 (Poly(ADP-ribose) polymerase 1) has a multifaceted function in the detection of DNA damage, including single-strand breaks (SSBs) and double-strand breaks (DSBs) via binding at the site of damage. PARP1 catalyzes the synthesis of poly(ADP-ribose) (PAR) chains using NAD⁺; the resulting PAR chains signal the recruitment of DNA repair proteins to the break, initiating the repair process. PARP1, as well as PARP2, are retained in nucleoli via interaction with the multifunctional nucleolar hub protein nucleophosmin, which is implicated in multiple steps of ribosome biogenesis, including rDNA transcription and elongation, as well as rRNA processing.

Method

HeLa cells were grown in a µ-Slide 8 Well high Glass-Bottom Ibidi chamber. DNA damage was induced via treatment with 2µM Etoposide-spiked complete growth media for 6 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human PARP1 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR018M), was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 5X in Identifyn's™ proprietary Wash Buffer, followed by the addition of Identifyn's™ proprietary STORM Buffer to the sample chamber, enabling image acquisition.

Control Data - Primary Unconjugated Antibody

Microscope

Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:

Calibration -

Calibration follows Identifyn's Calibration Standard Operating Procedure, which can be found here.

<https://identifyn.com/storm-calibration>

Image Acquisition -

SRX Software - Under the 'Single Molecule Localization' tab, 'Record' is selected and contains the following workflow tabs and parameters.

Configuration Tab - Capture Configuration

Number of Probes: 1
 Number of Simultaneous Probes: 1
 Number of Reference Probes: 1
 Camera: Both Cameras
 Color Channel: Both Channels
 Probe Capture Mode: Interleaved
 Z -Stack Capture Mode: Interleaved
 Multiple Location Capture: Not selected
 Post Record Reference: Not Selected
 Fluidics: Not Selected
 Autofocus Drift Correction: On

Microscope configuration

Biplane Module: 635nm Dichroic
 Objective: Silicon Selected
 Stage Insert: Rectangular Selected
 Course Focus Position: 8-Well Chamber Selected
 Dichroic: SuperRes
 PSF: (Red, Orange)
 Heater: Current: 26

Camera Configuration

Recording Vertical Field of View: 100%
 Widefield Camera Exposure Time: 100ms
 Super Resolution Camera Exposure Time: 20ms
 Widefield Camera Binning: 1
 Per-Probe Exposure Time: Selected

Probe Configuration

Predefined Probe: First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
 Photoactivation/Imaging Laser: 0% Selected
 Exposure Time: 20ms Selected

Experiment Configuration

Record Workflow: Selected Options
 View Mode: Widefield 200µm
 Capture Mode: Live

Stage and Piezo Selection

Examination of the Sample

Cell(s) or Cellular Structure Localization - The red-bordered box identifies the area of the SuperRes view and defines a suitable cell(s) or cellular structure. The cell(s) or structures are optimally focused.

Piezo Current: 181.0 μ m

Probe 1 Laser control (640 nm (635 nm Dichroic)

Widefield 640nm: 0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control (640 nm (640 nm Dichroic): Not Selected

Advanced Tools Option

Widefield camera Exposure Time: 100ms
Default values are maintained unless otherwise noted.

Widefield Laser: "Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected.

Autofocus - Calibration Values

Current Position" -0.979
Current Intensity: 4.5
Calibration Value: -5070 nm/unit
Autofocus Mode: Pre-Capture

View Mode

SuperRes: 50 μ m is selected

Capture Mode

Live

Stage and Piezo Selection

Cell(s) or Cellular Structure Localization - The target Cell or Subsection of the Target Cell is positioned and focused optimally.

Piezo Record: Begin: 180; End: 180.0

Probe 1 Laser control (640 nm (635 nm Dichroic)

SuperRes 640nm: 0.1X Neutral Density Filter: Selected, @0.02% Laser Input

Reference Probe 1 Laser control (640 nm (635 nm Dichroic)

Not Selected

Advanced Tools

Widefield Camera Exposure Time: 20ms
Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 20000

Z Positions: 1 using Steps: 0.2µm (200nm)

Cycles: 1

Time Points: 1

Pause Between Cycles: Not Selected

Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control (640 nm (640 nm Dichroic))

Laser Power: 10%

Expert Configuration Options - The Localization Tab

Beyond the values shown, all other parameters were set to their default values.

Localization

Cutout Width: 16px

Max Frame Accumulation: 1 frame

Max Accumulation Offset: 1px

Fiducial at Max Accumulation: Not selected

Max Particles Per Frame: No Max

Background SLPE Removal: Not Selected

Cutout/Background Removal: Not Selected

Preview Cutout Positions: Selected

Record Workflow - Localization Tab

Data Collection

The folder is created automatically with the localization file for this image acquisition.

Recording Summary

All parameters were carried over from previous tabs and are kept except:

Probe1: BG threshold: 7

Region of Interest -

Not selected

Display

Was deployed to inspect if the BG threshold applied chooses localizations in an expected manner

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting

Sizing Mode: Constant

Particle size: 20nm

Color by: Constant

Color Table: Single

Opacity Mode: Constant; Opacity 1: 0.8

Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)

Time Intervals: 6 Intervals

Pixel Size: 30nm

Smoothing: 8.00

Advanced Particle Filters - Applied to the drift-corrected data with the following parameters

PSF Z offset: -600 to +600

Radial precision: 30

Axial precision: 70

The other parameters were not applied.

Statistical analysis - Computed to clean low background signals.

Statistics Dialog - NA

Image by S. Thakur

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SOURCE PROVENANCE

Catalog	DC-000013
Stage	Single-molecule STORM
Instrument	Bruker Vutara VXL
Microscope configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Structured source metadata values	71
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

SOURCE PROVENANCE

Cell model	HeLa
Image SHA-256	86E0AEB84093D2962FBD4A7CA8F69006769ED13B054BA1F778991F6EB46C89AF
Method PDF SHA-256	D8DD6198B42ECE63DC44BA6FCEA7144A236F24A786DF2E7EE3BAE1391C9CEB79